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Published in:
FEBS Journal

DOI:
[10.1111/febs.13999](https://doi.org/10.1111/febs.13999)

Publication date:
2017

Document Version
Peer reviewed version

[Link to publication in Discovery Research Portal](#)

Citation for published version (APA):

Dayalan Naidu, S., & Dinkova-Kostova, A. T. (2017). Regulation of the mammalian heat shock factor 1. *FEBS Journal*, 284(11), 1606-1627. <https://doi.org/10.1111/febs.13999>

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Regulation of the mammalian heat shock factor 1

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Abbreviations:

17-AAG, 17-allylamino 17-demethoxygeldanamycin; ATF, activating transcription factor; CDK, cyclin-dependent kinase; CTAD, C-terminal transactivation domain; DBD, DNA-binding domain; 15d-PGJ₂, 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂; FBXW, ERK, extracellular signal-regulated kinase; F-box/WD repeat-containing protein; FILIP-1L, filamin interacting protein 1-like; GSK3, glycogen synthase kinase 3; HAT, histone acetyl transferase; HDAC, histone deacetylase; HSF, heat shock factor; Hsp, heat shock protein; HSR, heat shock response; IL, interleukin; JNK, c-Jun N-terminal kinase; LZ, leucine zipper; MAPK, mitogen-activated protein kinase; MEF, mouse embryonic fibroblasts; mTOR, mechanistic target of rapamycin; NEDD4, neural precursor cell expressed developmentally down-regulated protein 4; NRF2, nuclear factor-erythroid 2 p45-related factor 2; nSB, nuclear stress bodies; PTM, post-translational modifications; RHT, rocaglate, heat shock, initiation of translation inhibitor; RD, regulatory domain; SCF, Skp, Cullin, F-box containing complex; SIRT, sirtuin; TAD, transactivation domain.

Keywords: cytoprotection; hormesis; HSF1 activator; HSF1 inhibitor, phytochemical; sulfhydryl reactivity

Abstract

Living organisms are endowed with the capability to tackle various forms of cellular stress due to the presence of molecular chaperone machinery complexes that are ubiquitous throughout the cell. During conditions of proteotoxic stress, the transcription factor heat shock factor 1 (HSF1) mediates the elevation of heat shock proteins, which are crucial components of the chaperone complex machinery and function to ameliorate protein misfolding and aggregation and restore protein homeostasis. In addition, HSF1 orchestrates a versatile transcriptional program that includes genes involved in repair and clearance of damaged macromolecules and maintenance of cell structure and metabolism, and provides protection against a broad range of cellular stress mediators, beyond heat shock. Here, we discuss the structure and function of the mammalian HSF1, and its regulation by post-translational modifications (phosphorylation, sumoylation, and acetylation), proteasomal degradation, and small molecule activators and inhibitors.

Introduction

In the early 1960s, the Italian researcher Ferruccio Rittosa was studying the type(s) of nucleic acid that was being transcribed at the puffs in the chromosomes of the *Drosophila* salivary glands, where these chromosome puffs were found to be transcriptionally active regions [1]. By accident, one of his colleagues had increased the temperature of the incubator where he had kept his *Drosophila* larvae and during this time, Ritossa noticed a unique puffing pattern in the *Drosophila* polytene chromosomes when they were exposed to heat, sodium salicylate or dinitrophenol [2]. Subsequently, independent groups observed this phenomenon in other *Drosophila* tissues, and this ultimately led to the discovery of the heat shock proteins when Tissieres and colleagues found these proteins to be upregulated during the chromosomal puffing that occurred upon heat stress [3].

In all living organisms, the cellular response to elevated temperatures is universal, and is triggered by a temperature increase of just a few degrees above the optimal temperature. Cells within these organisms possess intricate mechanisms that allow for adaptation and survival during elevated temperatures. In humans, temperatures above 41-42 °C induce the heat shock response (HSR), which is characterized by the activation of transcription of *heat shock protein (Hsp)* genes orchestrated by a family of transcription factors, within which heat shock factor 1 (HSF1) is most prominent. The HSR causes the elevation of heat shock proteins such as Hsp27, Hsp40, Hsp70 and Hsp90, most of which are molecular chaperones that function to prevent protein misfolding and aggregation within the cell. The HSR is a cytoprotective mechanism that is also stimulated upon cellular stresses such as hypoxia [4], fluctuations in intracellular pH [5], and exposure to heavy metals [6, 7].

In addition to providing an adaptive response to thermal stress resulting in thermotolerance, the HSR orchestrated by HSF1 has protective effects in numerous pathophysiological conditions, including ageing and neurodegenerative diseases [8]. This is because HSF1 has a large number of target genes encoding proteins with versatile cytoprotective functions. Global transcriptional profiling, differential display, and proteomic approaches have revealed that, in different organisms, approximately 50–200 genes are induced by heat shock [9]. These include the classical molecular chaperones that prevent unspecific aggregation of non-native or partially misfolded proteins, proteolytic proteins that can eliminate or recycle irreversibly-damaged

proteins which cannot be refolded by the chaperones, RNA- and DNA-modifying enzymes which participate in DNA damage repair, proteins involved in sustaining cellular structures such as the cytoskeleton and membranes, and proteins which participate in transport and detoxification. In addition, heat shock upregulates a number of metabolic enzymes that are needed to reorganize and maintain the energy supply of the cell. Induction of other transcription factors, kinases and phosphatases also occurs following heat shock, and may further activate other stress response pathways, thus amplifying the initial signal.

Notably, not all genes are induced at the same time and with the same duration: some, such as those responsible for the correct protein folding, are immediate responders (within minutes), whereas others, such as those involved in DNA damage repair and cell metabolism, are somewhat slower (within hours) [9]. Overall, induction of the HSR provides broad protection against stress of various different types, including and extending beyond heat shock. A study in the nematode *C. elegans* has shown that activation of Hsp90 in response to an imbalance in proteostasis in one tissue functions in a non-autonomous fashion termed transcellular chaperone signaling to initiate a protective response in adjacent tissues and restore the protein homeostasis within the whole organism [10]. In agreement, excitation of serotonergic neurons in *C. elegans* with the consequent release of serotonin is sufficient to activate HSF1 and the HSR, and to suppress protein misfolding in peripheral tissues even in the absence of temperature increase [11]. It will be important to determine whether similar signaling mechanisms operate in mammals.

HSF Family Members

To date, four members belonging to the HSF protein family have been identified, of which HSF1, HSF2 and HSF4 [12, 13] have been characterized in mammals, whereas HSF3 was initially identified in the avian species [14]. The human, mouse and bovine HSF1 share approximately 84% sequence identity (**Fig. 1** and **Fig. 2**). The mammalian HSF2 and HSF4 are also well conserved across species, sharing approximately 88% and 79% amino acid sequence identity, respectively. In humans, the amino acid sequence of HSF1 shares 32% and 36% sequence identity to HSF2 and HSF4, respectively.

In unstressed cells, HSF1 is a monomer and in contrast, HSF2 is a dimer. Upon stress, HSF2 can either form a homotrimer or it can heterotrimerize with HSF1 in the nucleus and induce the transcription of both the classical heat shock genes as well as non-classical heat shock genes such as the tandem satellite III DNA repeats [15, 16]. A recent study has discovered that HSF1 and HSF2 interact with each other through their coiled-coil domains adjacent to their DNA binding domains [17]. Unlike HSF1, HSF2 expression is tissue- and cell-specific [18]. In the mouse, HSF2 plays a vital role in spermatogenesis, female fertility and early development [19, 20]. Kallio et al. discovered that HSF2-knockout (HSF2^{-/-}) mice, although embryonically viable, display brain abnormalities, which are characterized by a reduced striatum and hippocampus; furthermore, HSF2^{-/-} female mice produce eggs with meiotic defects [20]. Although much of the early research on HSF2 was focused on its role in development, a study by Shinkawa et al. [21] has highlighted the link between HSF2 and neurodegeneration. These researchers found that loss of HSF2 in the R6/2 Huntington's disease mouse model exacerbates the aggregation of the polyglutamine protein leading to decreased lifespan, partly through increased expression of the small heat shock protein α B-crystallin.

HSF4 lacks the LZ4 domain (see below) that is required for the suppression of trimerization of HSF1, is localized mainly in the nucleus, and is ubiquitously expressed. HSF4 plays an important role in development and its expression is increased during lens development [22]. Many studies have shown that HSF4 is crucial for the development and maintenance of the lens as mutations in HSF4, particularly in the DNA-binding domain, are correlated with the development of cataracts in humans [23-26]. One of the isoforms of HSF4, HSF4a, has been shown to negatively regulate HSF2 by downregulating the expression and inhibiting the transcriptional activity of HSF2 at the *hsp70* promoter via direct binding to HSF2 [27]. Loss of the ATP-dependent chromatin remodelling enzyme Snf2h is vital for lens development in mice. Interestingly, depletion of Snf2h dramatically reduces HSF4 transcript levels suggesting that the transcription factor is regulated by chromatin remodelling, and that the role of Snf2h in lens development might be in part mediated by HSF4 [28].

The mammalian HSF1 ortholog in avian cells is functionally redundant, however in the chicken, HSF3 is the major heat shock transcription factor [29]. Furthermore, human HSF1 is able to rescue the induction of the classical heat shock

responsive genes in the absence of chicken HSF3, although chicken HSF1 confers cellular cytoprotection when cells are exposed to stressors such as ionizing or ultraviolet irradiation [30]. Interestingly, this occurs without inducing the classical heat shock genes, suggesting that chicken HSF1 also plays a role in other pro-survival pathways. In the chicken, HSF3 exists as a dimer in unstressed cells and is able to form homotrimers upon heat shock [14, 31]. The mouse HSF3 discovered in 2010 by Fujimoto et al. [32] can activate the transcription of non-classical heat shock genes in HSF1-knockout mouse embryonic fibroblasts (MEF) cells, and its isoform mHSF3a, has the ability of protect cells from heat shock and proteotoxicity. Of note, although HSF3 is considered to be the avian ortholog of the mammalian HSF1, one study suggests its involvement in the regulation of a number of febrile response mediators, such as the interleukins IL-6, IL-1 β , and activating transcription factor 3 (ATF3) [33].

HSF1

HSF1 is the most well studied member of the HSF family. During unstressed conditions, the mammalian HSF1 exists as a monomer more abundantly in cytoplasm relative to the nucleus (**Fig. 3**). The DNA-binding and transcriptional capacity of HSF1 is suppressed at the intramolecular level as well as the intermolecular level, where in the latter, molecular chaperones such as Hsp70 [34], Hsp90 [35], and TRiC/CCT [36] interact with HSF1 to inhibit its activation. Upon cellular stress, HSF1 undergoes several activating post-translational modifications and forms a transcriptionally active trimer that accumulates in the nucleus and binds to heat shock elements (HSE), inverted repeats of the pentameric sequence nGAAn that are found in the upstream regulatory regions of the *hsp* genes [37]. By use of fluorescence polarization and thermal denaturation profiling combined with quantitative chromatin immunoprecipitation assays, Jaeger et al. [38] have demonstrated a role for specific orientations of extended HSE sequences in driving preferential HSF1 DNA binding to its target loci.

Nuclear stress bodies (nSB) are relatively large distinct structures (0.3 to 3 μ m in diameter) that are formed in the nucleus when the cell is exposed to stress such as heat shock or heavy metals [39]. The formation of nSB requires the transcriptionally active HSF1 to interact with pericentromeric tandem repeats of satellite III sequences within the 9q12 chromosomal locus leading to the transcription of non-coding single-

stranded RNA molecules, which have been proposed to function by recruiting specific factors and affecting chromatin organization. Also, HSF1 binds to the histone acetyl transferase (HAT) CREB and causes the hyperacetylation of nucleosomes at the satellite III repeats, allowing RNA polymerase as well as several RNA-binding proteins to transcribe sat III mRNA transcripts. The mRNA and RNA-binding protein complexes generated are known as the perichromatin granules and are generated in large clusters during stress forming mature distinct nSB [40]. Interestingly, at the nSB, HSF1 and HSF2 form transcriptionally active heterocomplexes [41], and the formation of nSB coincides with the nucleolar accumulation of Hsp70. Although, the function of the nSB is currently not clear, it is noteworthy that upon stimulation, nSB are formed as early as 5 minutes and they are able to persist for several hours after the initial stimulation. During the heat shock response, Hsp70 levels are increased, and the HSF1 trimers that exit the nSB are dissociated into inactive HSF1 monomers by the binding of Hsp70 [40].

The generation of HSF1-knockout mice (HSF1^{-/-}) provided important insights into the functional significance of HSF1 [42, 43]. HSF1^{-/-} mice have altered redox homeostasis in the cardiac cells, and their mitochondria are highly susceptible to oxidative damage. It was found that HSF1 is essential for induction of the classical heat shock genes, and HSF1^{-/-} MEF cells are more sensitive to apoptosis during heat stress compared to their wild-type counterparts, showing the importance of HSF1 for cell survival. Female HSF1^{-/-} mice display placental insufficiency, which contributes to the partial fetal lethality of the absence of the transcription factor. Male HSF1^{-/-} mice are fertile, however the females with this genotype are sterile. HSF1 plays a vital role in oogenesis: HSF1 is highly abundant in the nucleus of immature oocytes and plays an important role in the initial cleavage stages, hence explaining the infertility described in the HSF1^{-/-} females [44].

Post-translational Modifications of the HSF1 protein

HSF1 is constitutively expressed in most tissues and cell types and is regulated by multiple post-translational modifications (PTM) such as sumoylation, acetylation and phosphorylation (**Fig. 4**) throughout its activation and attenuation cycle. In an unstressed system, HSF1 is a monomeric phosphoprotein where it is phosphorylated on multiple serine residues [45-48], and is also negatively regulated at the

intramolecular level due to the binding of the leucine zipper (LZ)1-3 domain and the LZ4 domain. The LZ1-3 domain, or the oligomerization domain, due to its coiled-coil structure has the ability to form homotrimers [49-51]. Upon stress, the intramolecular coiled-coil interaction is lost and an intermolecular coiled-coil interaction is established. In addition, phosphorylation on serine 303 that lies within the regulatory domain of HSF1 is required for sumoylation [conjugation of small ubiquitin modifiers (SUMO) 2/3] at lysine 298 [52], and this mechanism was first described in HSF1, where phosphorylation-dependent sumoylation motif (PDSM) characterized by the consensus sequence $\Psi KxExxSP$ (where Ψ is a branched hydrophobic amino acid and x is any amino acid) was identified [53]. Sumoylation at lysine 298 renders HSF1 transcriptionally incompetent [54]. Phosphorylation of serine 303 and 307 itself does not affect the transcriptional activity of HSF1; however, the phospho-S303-dependent sumoylation on lysine 298 blocks its transactivation capacity [55].

The DNA-binding domain (DBD) (**Fig. 1**, **Fig. 4** and **Fig. 5**) located at the N-terminal region of HSF1 is highly conserved across species [8]. Within the HSF1 trimers, each of the DBD of the monomers recognizes the nGAAn sequence in the major groove [56, 57]. Phosphorylation of serine 121 by mitogen-activated protein kinase (MAPK)-activated protein kinase 2 (MAPKAPK2) at the DBD causes HSF1 to lose its transcriptional activity and promotes binding to its negative regulator Hsp90 [58]. In addition, acetylation on lysine 80 hinders the transcriptional capacity of HSF1, as mutagenesis studies have revealed a loss-of-function phenotype when lysine 80 was mutated [59-61]. Activation of the deacetylase sirtuin-1 (SIRT1) prolongs the transcriptional competency of HSF1, whereas the decrease in SIRT1 accelerates the attenuation phase of the HSR [61].

The regulatory domain (RD) of HSF1 lies between the LZ1-3 and LZ4 domains. Its absence causes HSF1 to become transcriptionally active even in unstressed conditions, which highlights the role of the RD as a repressor of HSF1 activity and also as a stress sensor [62, 63]. The RD is subjected to many PTM such as acetylation, phosphorylation and sumoylation (**Fig. 4**). In 2005, Guettouche and colleagues performed an extensive study on the phosphorylation sites on human HSF1 and identified that HSF1 was phosphorylated on multiple serine residues, and interestingly, they did not detect phosphorylation on threonine or tyrosine residues [64]; however, other groups have identified phosphorylation events occurring on such residues [65, 66]. Most phosphorylation modifications within the RD are inhibitory,

and a mutant HSF1, where the 15 known phosphorylation sites within the RD are disrupted, is a potent transactivator of its target genes, with reduced activation threshold [67]. However, phosphorylations on serine 230 [47] and serine 326 [64, 68] are activating. Thus, the calcium/calmodulin-dependent kinase CAMKII phosphorylates HSF1 at serine 230 during stress conditions, and mutation of this residue reduces the transcriptional capacity of HSF1 [47]. The phosphorylation of serine 326 of HSF1 allows DAXX to bind to HSF1 to enhance its transcription [68], and mutations of serine 326 have been reported to reduce the transcriptional activity of HSF1 [64, 68]. Several kinases have been implicated in phosphorylating HSF1 at serine 326, including the mechanistic target of rapamycin (mTOR) [69], the mitogen-activated protein kinase kinase MEK [70] and the p38 MAPK [71]. Notably, among the p38 MAPK family members, p38 δ MAPK is a particularly efficient catalyst of this phosphorylation, whereas p38 γ MAPK is the most specific [71].

The available literature on the functional characterization of the HSF1 C-terminal transactivation domain (CTAD) is limited compared to the other domains described above. The CTAD consists of two transactivation domains, TAD1 and TAD2 [63]. TAD1 (amino acids 401-420) is rich in hydrophobic amino acids and interacts with TATA-box binding protein associated factor TAF9 *in vitro*, and mutations within the hydrophobic patch of TAD1 prevent HSF1 transactivating capability [72]. TAD2 contains both acidic and hydrophobic residues and is proline-rich. BRG1 belonging to chromatin remodeler SWI/SNF complex binds to HSF1 at the CTAD [73]. It is likely that the CTAD could be the hub for interaction of transcriptional co-factors and HSF1. Interestingly, Hsp70 binds to the CTAD of HSF1 [74], therefore, it could potentially negatively regulate HSF1 by disrupting the association of HSF1 with its transcriptional co-activators such as p300/CBP [75]. Although it is clear from the literature that HSF1 and Hsp90 interact [34, 35, 76], to our knowledge, the precise interaction site has not been mapped.

HSF1 Protein Turnover

The endogenous HSF1 protein has a relatively long half-life, in the range of 13.6 h to 20 h [77-79]. Unlike the long half-life of the protein, the half-life of HSF1 mRNA is approximately 100 min [80]. It is highly likely that the reason for HSF1 being such a long-lived protein is to be readily available in the event of exposure to cytotoxic stress

so that it is able to exert its cytoprotective effects immediately. The histone acetyltransferase E1A Binding Protein P300 (EP300) promotes the stability of endogenously expressed HSF1 through acetylation on several of its lysine residues (i.e., lysine 208 and lysine 298) hence preventing it from being ubiquitinated and targeted for proteasomal degradation [81]. EP300 maintains HSF1 stability irrespective of its phosphorylation status [81].

One of the first insights regarding HSF1 turnover was provided by Bonelli and colleagues who found that only under heat shock conditions, serum-starved late passage human fibroblast cells displayed reduced HSF1 levels compared to early passage serum-starved cells [82]. This finding is further supported by a recent publication showing that HSF1 degradation increases dramatically post-heat shock and furthermore, RNAi-mediated depletion of the proteasomal subunit PSMA7, prolonged the Hsp70 mRNA production by approximately 5 h under conditions of heat shock suggesting that the HSR is attenuated by the proteasome [81]. Interestingly, riluzole which is the only FDA-approved drug for the treatment of the neurodegenerative disease amyotrophic lateral sclerosis, is able to dramatically increase the protein half-life of endogenous HSF1 from 13.6 h to 69.3 h [78], allowing greater activation of the HSR in cancer and neuronal cells [78, 83]. Furthermore, silencing LAMP2A using RNAi, curbed the turnover HSF1 in the presence of riluzole which suggests that the compound could potentially block the clearance of HSF1 through chaperone-mediated autophagy as LAMP2A is an essential potentiator of this process [78].

Until recently, not much was known about the mechanisms by which HSF1 is degraded, however, studies have emerged implicating several proteins that mediate the degradation of HSF1, namely, filamin interacting protein 1-like (FILIP-1L) and the E3 ligases, SCF β /TrCP, F-Box and WD repeat domain containing protein 7 (FBXW7) and neural precursor cell expressed developmentally down-regulated protein 4 (NEDD4). FILIP-1L was identified as a binding partner of HSF1 through a yeast-two-hybrid screen [84]. The downregulation of FILIP-1L has been implicated in various human tumours and has been linked with hypermethylation at the *FILIP-1L* gene promoter and to global DNA methylation [85, 86]. Hu and Mivechi showed that overexpression of FILIP-1L leads to the decrease in HSF1 levels and as a consequence inhibits the transcription of HSF1 target genes. They further found that FILIP-1L acts as an adaptor protein for HSF1 to allow for binding to hHR23A, an

ubiquitin receptor protein that functions to transfer ubiquitinated proteins to the 19S proteasome [84].

As described in the previous section, phosphorylation of HSF1 at serine 216 targets the protein for degradation by the E3 ubiquitin ligase SCF/ β -TrCP during mitosis, and mutation of this residue to arginine, dramatically increases its half-life [87]. FBXW7 is the substrate recognition component of the SCF ubiquitin ligase complex and in humans it functions as a tumour suppressor as it mediates the degradation of many proto-oncogenes such as MYC, NOTCH and JUN [88]. FBXW7 binds to its substrates via their phosphodegron motifs which are called Cdc4 phosphodegrons, where phosphorylation within the phosphodegrons has to occur in order for binding to take place [88]. In most cases, FBXW7 substrates such as the ones mentioned above are phosphorylated by GSK3 within the phosphodegron. Interestingly, a recent article has linked the protein stability of HSF1 to FBXW7 α ; the authors reported that the SCF/FBXW7 complex targets the degradation of HSF1 through direct binding at the phosphodegron motif spanning serine 303 and serine 307, where GSK3 and ERK1 phosphorylate these sites respectively [79]. The phosphorylation of serine 303 and serine 307 is required for the binding of FBXW7 α to HSF1, and loss of FBXW7 α causes persistent nuclear accumulation and activation of HSF1. In human melanoma cells, FBXW7 α is downregulated, and Kourtis and colleagues showed that HSF1 accumulation and activation drives the metastatic potential [79]. GSK3 is activated under low-serum or serum-starved conditions [89, 90]. Taken together, the observations by Bonelli et al. and Kourtis et al. suggest that during serum starvation, heat shock could cause the activation of GSK3 and subsequent phosphorylation of HSF1 at the serine 303 and serine 307 phosphodegron to allow for SCF/FBXW7-mediated degradation of the transcription factor [79, 82].

Numerous reports have linked the loss of HSF1 to the promotion of neurodegenerative diseases [91, 92]. α -Synucleinopathies represent progressive neurodegenerative diseases such as Parkinson's disease, multiple system atrophy and dementia with Lewy bodies [93, 94] where there is accumulation of α -synuclein in the cytoplasm of a subset of neuronal and glial cells. A53T α -synuclein is able to form aggregates more readily than WT α -synuclein. In a recent study, Kim et al. found that overexpression of the mutant A53T α -synuclein in neuronal cells caused a decrease in HSF1 protein levels of more than 70% compared to WT α -synuclein whilst not affecting the HSF1 mRNA levels [95]. WT α -synuclein caused the decrease of HSF

levels in the nucleus unlike the mutant A53T α -synuclein which decreased HSF1 levels in both the nucleus and cytoplasm [95]. Furthermore, A53T α -synuclein overexpression increased the amount of ubiquitinated HSF1. Because the authors had previously identified the E3 ligase NEDD4 levels to be elevated in brains from Parkinson's disease patients [96], they overexpressed NEDD4 in neuronal cells and observed a prominent decrease in HSF1 protein levels [95]. Conversely, loss of NEDD4 or the overexpression of HSF1 was neuroprotective against α -synucleinopathy [95].

Small Molecule Activators of HSF1

Numerous small molecules, both endogenously occurring as well as of exogenous origin, have been shown to activate HSF1 (see West et al. [97] for a comprehensive review). In most cases, these compounds are inducers of the transcriptional activity of HSF1. Here we give examples of endogenous metabolites as well as phytochemicals, which have been isolated from edible and/or medicinal plants, and are contributing to the health-promoting effects of plant-rich diets. We then describe the synthetic HSF1 activators, which have been identified using reporter systems and high-throughput screening strategies. The chemical properties of these compounds have provided important insights into the mechanisms of activation of the HSR. In particular, sulfhydryl reactivity, a characteristic feature of many HSF1 activators, underlies their ability to affect the function of HSF1 and some of its critical regulatory proteins, such as Hsp90, SIRT1, or upstream regulatory kinases [98].

Endogenous metabolites

HSF1-mediated transcription is induced by endogenously produced electrophilic oxidized and nitrated lipids, as well as α,β -unsaturated aldehydes. Examples include 4-hydroxy-2-nonenal, acrolein, 10-nitro-octadecenoic acid (nitro-oleic acid), and 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂ (15d-PGJ₂) (**Fig. 6**). Nuclear accumulation of HSF1 and induction of Hsp70 and Hsp40, as well as of HSE-dependent luciferase reporter, have been observed when human colon cancer cells were exposed to 4-hydroxy-2-nonenal, and siRNA-mediated silencing of HSF1 abolished this induction [99]. Treatment of human endothelial cells with 10-nitro-octadecenoic (nitro-oleic) acid, a nitrated product of oleic acid with cytoprotective activities, led to activation of the heat shock

response [100]. Induction of Hsp70 and Hsp40 also occurred when A549 human lung cancer cells were exposed to the electrophilic lipid peroxidation product acrolein [101]. The DNA binding activity of HSF1 was increased and the expression of Hsp70 was robustly upregulated by 15d-PGJ₂ in the heart of male Wistar rats undergoing ischemia-reperfusion injury [102]. Interestingly, high concentrations of 17 β -estradiol have been shown to activate HSF1 and induce Hsp70 [103, 104]. Unlike the other endogenous HSF1 activators mentioned above, 17 β -estradiol is not electrophilic; however, it can be metabolically converted to the electrophilic quinone derivatives 2-hydroxy- and 4-hydroxy-estradiol [105], which might be the ultimate inducers.

In addition to endogenous electrophiles, the HSF1-mediated HSR is directly activated by the oxidant hydrogen peroxide (H₂O₂). Interestingly, in this case, HSF1 itself is the protein sensor as activation of murine HSF1 by H₂O₂ is dependent on cysteine 35 and cysteine 105, which form a disulfide bridge [106]. The corresponding pair of cysteines (i.e., cysteine 36 and cysteine 103) in human HSF1 form an intermolecular disulfide bridge, promoting HSF1 trimerization and binding to the HSE [107]. A subsequent study has shown that an intermolecular interaction between the aromatic residues tryptophan 37 and phenylalanine 104 supports the approach of cysteine 36 and cysteine 103 [108]. In contrast, an intramolecular disulfide bridge formation (in which cysteine 153, cysteine 373 and cysteine 378 participate) inhibits trimerization and DNA binding of HSF1 [107]. The signalling molecule nitric oxide has been reported to activate HSF1 and induce Hsp70 in vascular smooth muscle cells [109]. Interestingly, *S*-nitrosation at cysteine 597 of human Hsp90 α inhibits the ATPase activity of the chaperone [110], and substituting cysteine 597 with *S*-nitrosation-mimicking residues, such as asparagine and aspartic acid, shifts the conformational equilibrium of the chaperone, decreasing its activity [111]. It is therefore possible that HSF1 activation by nitric oxide is due to inactivation of Hsp90.

Activation of HSF1 can also be stimulated by electrophilic and reactive oxygen species which are formed during physiological and pathophysiological processes. Thus, HSF1 is activated in atherosclerotic lesions, and cytokine stimulation and mechanical stretching of smooth muscle cells result in HSF1 hyperphosphorylation, nuclear translocation, and enhanced Hsp70 expression [112]. In addition, HSF1 activation has been implicated in the production of plasminogen activator inhibitor-1 after stimulation by glycated low density lipoproteins as well as

by oxidized very low density lipoproteins in cultured vascular endothelial cells [113, 114]. Subsequent studies have shown that in this case, activation of HSF1 is mediated by induction of NADPH oxidase, implicating superoxide as the ultimate activator [115, 116].

Phytochemicals

A screen of bioactive small molecules in the human cervical cancer HeLa cell line that is stably transfected with a luciferase-encoding construct under the transcriptional control of the *HSP70* promoter has identified the phytochemical celastrol as inducer of the HSR [117]. Celastrol is a quinone methide triterpenoid (**Fig. 7**) found in the Chinese plant *Tripterygium wilfordii*. In addition to the HeLa reporter cell line, celastrol activates the *hsp70* promoter reporter in the breast cancer cell lines MCF7 and BT474, the nonsmall cell lung carcinoma cell line H157, and the neuroblastoma cell line SH-SY5Y), and importantly, the magnitude of activation is comparable to that induced by heat shock (42°C) [117]. In agreement, exposure to celastrol is protective against lethal heat stress, and to a similar extent as a 42 °C heat shock. Treatment with celastrol leads to hyperphosphorylation of HSF1, enhanced binding of HSF1 to the HSE, and transcriptional activation of endogenous heat shock genes [117]. Subsequent studies have suggested that inhibition of Hsp90 by celastrol may be the initial event that triggers dissociation of HSF1 from the protein complex [118, 119]. This possibility is further supported by mechanistic studies showing that celastrol reacts with Hsp90 and inhibits the ATPase activity of the chaperone without affecting ATP binding [71, 120].

The tetranortriterpenoid gedunin (**Fig. 7**) from the Indian neem tree *Azadirachta indica* is another activator of HSF1. Compared to celastrol, gedunin is less potent, but appears to use similar mechanisms by which it activates HSF1, i.e. inhibition of the function of the negative regulator Hsp90 [119, 121]. The use of a high content screening platform, an image-based, multiparametric assay which monitors the formation of HSF1/Hsp70 stress granules in heat-shocked HeLa cells, identified four compounds closely related to gedunin, i.e., deoxygedunin, deacetoxy-7-oxogedunin, deacetylgedunin, and sappanone A, which were subsequently confirmed to activate HSF1 and induce Hsp70 in an HSF1-dependent manner [122].

Withaferin A (**Fig. 7**), a withanolide found in the Indian plant *Withania somnifera*, was identified in a screen of a library of more than 80,000 natural and

synthetic compounds using a reporter cell line which expresses enhanced green fluorescent protein (EGFP) under the transcriptional control of a minimal consensus HSE-containing promoter [123]. Like celastrol and gedunin, withaferin A also inhibits the function of Hsp90 [124, 125], and this inhibition may be responsible for activation of HSF1. The same high-throughput screen confirmed that celastrol, withaferin A, gedunin are all inducers of the HSR, and identified several other natural products, such as the limonoids anthothecol, cedrelone, and the gedunin derivative 7-desacetoxy-6,7-dehydrogedunin, as well as a fungal product, the macrocyclic lactone dehydrocurvularin among the active compounds [123]. Notably, all of these compounds have an α,β -unsaturated carbonyl functional group, emphasizing the importance of electrophilicity for inducer activity.

Curcumin [1,7-bis(4-hydroxy 3-methoxy phenyl)-1,6-heptadiene-3,5-dione, diferuloylmethane, **Fig. 7**], a polyphenol from the East Indian plant *Curcuma longa*, has been shown to induce heat shock proteins in numerous cell culture and animal models [126, 127]. The observed disruption of the binding of the Hsp90-p23 complex to its client protein p210 BCR/ABL, and the consequent degradation of the kinase in chronic myelogenous leukemia cells upon exposure to curcumin [128] suggests that, for this compound too, induction of heat shock proteins could be a consequence of inhibition of Hsp90 function. Notably, curcumin has been present in the human diet for centuries, and there is a wealth of information regarding its safety and efficacy in both animals and humans [129]. To date, more than 100 clinical trials have been registered to evaluate this polyphenol in a number of conditions, including mild cognitive impairment, Alzheimer's disease, multiple myeloma, pancreatic cancer, colorectal cancer, and myelodysplastic syndrome (www.clinicaltrials.gov).

Coniferyl aldehyde (**Fig. 7**) is a phenolic compound, which is found as a glucoside in the bark of *Eucommia ulmoides*, a plant that is used in the traditional medicine of Korea, Japan, and China. Coniferyl aldehyde has been shown to increase the protein stability of HSF1, activate the mitogen-activated protein kinases extracellular signal-regulated kinases (ERK)1/2, c-Jun N-terminal kinase (JNK)1, and p38, promote HSF1 phosphorylation at serine 326, and consequently upregulate the expression of Hsp27 and Hsp70 [130]. Furthermore, the same study showed that coniferyl aldehyde treatment protected cells and mice against damage induced by ionizing radiation or taxol, but was not effective in HSF1^{-/-} cells, demonstrating the essential requirement for HSF1 for the protective activity of this phytochemical [130].

Another aromatic phytochemical, the chalcone derivative (\pm)-4,2',4'-trihydroxy-3'-[(6E)-2-hydroxy-7-methyl-3-methylene-6-octenyl]chalcone (**Fig. 7**), was also reported to activate HSF1-dependent transcription in human lung cancer NCI-H460 cells [131]. In an effort towards identification of heat shock protein-inducing agents from natural products, this chalcone was isolated from the areal parts of *Angelica keiskei* Koidzumi (Umbelliferae), an edible plant that is considered health food in Korea and Japan [131].

Sulforaphane [1-isothiocyanato-(4*R*)-(methylsulfinyl)butane, **Fig. 7**] is an isothiocyanate which is formed from a glucosinolate precursor (glucoraphanin) upon plant tissue injury. Importantly, cruciferous vegetables, such as broccoli (*Brassica oleracea*), are rich dietary sources of this phytochemical. Nuclear accumulation of HSF1 and induction of heat shock proteins by sulforaphane treatment has been demonstrated in several cell culture models, including human cell lines, as well as in animals after a single oral dose of the isothiocyanate [132-134]. Another isothiocyanate, phenethyl isothiocyanate (**Fig. 7**), which occurs in watercress (*Nasturtium officinale*), has also been shown to induce expression of heat shock proteins in cultured cells and animals [71, 135]. Phenethyl isothiocyanate inhibits Hsp90, activates p38 MAPK, increases the phosphorylation of HSF1 at serine 326, and robustly upregulates HSE-mediated transcription of endogenous as well as reporter genes [71]. Similarly, the levels of Hsp70 are robustly upregulated upon exposure of cells to the aromatic benzyl isothiocyanate, but not the aliphatic allyl isothiocyanate [71].

The available experimental evidence points to Hsp90 as the main intracellular target of the isothiocyanates to facilitate HSF1 activation: (i) co-treatment with sulforaphane enhances the anti-tumor effect of the Hsp90 inhibitor 17-allylamino 17-demethoxygeldanamycin (17-AAG) [136]; (ii) sulforaphane disrupts the interaction of Hsp90 with its co-chaperone Cdc37 [136]; (iii) synergistically with 17-AAG, the isothiocyanate downregulates several Hsp90 client proteins, such as mutant p53, Raf-1, and Cdk4 [136], (iv) Hsp90 is covalently modified by a sulfoxythiocarbamate derivative of sulforaphane both *in vitro* and in cells [137-139], (v) the fraction of Hsp90-bound HSF1 is smaller in lysates from cells that had been treated with phenethyl isothiocyanate compared to solvent-treated cells, suggesting dissociation of HSF1 from Hsp90 [71], and (vi) the ability of sulforaphane to downregulate the activity of histone deacetylase (HDAC) [140-144] may further inhibit the activity of

Hsp90 through altering acetylation of the chaperone. Indeed, inhibition or knockdown of HDAC6 leads to acetylation of Hsp90 and disruption of its chaperone function [145], and treatment with sulforaphane downregulates the activity of HDAC6, resulting in hyperacetylation of Hsp90 [144].

In contrast to the electrophilic phytochemicals discussed above, the stilbene resveratrol (**Fig. 7**) appears to activate HSF1 by promoting the persistent DNA binding of the transcription factor and suppressing the attenuation phase of the HSR [61]. In this case, the phytochemical functions by activating the NAD^+ -dependent deacetylase SIRT1 and thus maintaining HSF1 in a deacetylated, DNA-binding competent state. Resveratrol directly activates SIRT1 through an allosteric mechanism resulting in the lowering of the K_m for both the acetylated protein substrate and for the cofactor NAD^+ [146]. Most small-molecule SIRT1 activators known to date are planar molecules comprised of multiple phenyl rings bearing hydroxyl groups [146]. Whether other phytochemicals which are known to activate SIRT1, such as the flavonol fisetin and the chalcone butein [147], could have a similar effect on HSF1 is presently unknown.

An important aspect of the biological activities of many of the phytochemicals discussed here is that exposure of cells and organisms to these compounds often results in non-linear dose responses. This is in line with the concept of hormesis, which is characterized by a low-dose stimulation and a high-dose inhibition, resulting in either a J-shaped or an inverted U-shaped dose response [148, 149]. Ingestion of phytochemicals such as curcumin can initiate mild cellular stress involving free radical production, ion fluxes, and increased energy demand [150, 151]. This is followed by activation of adaptive stress response pathways, such as those regulated by HSF1, leading to transcriptional induction of cytoprotective proteins, and ultimately enabling cell survival. Importantly, once mounted, the hormetic response is protective against more severe stress conditions, which otherwise may be lethal. In addition, this protection is not limited to higher doses of the same stress agent, but extends to other types of stressors, including oxidative, metabolic, and thermal stress.

Synthetic compounds

The possibility of developing small molecule activators of the HSR as pharmacological agents for protection against human diseases, especially protein conformational diseases, has led to the development of several high-throughput screening strategies. Neef et al. have reported the development of a humanized yeast-based high-throughput screen, which is insensitive to proteotoxic stress and Hsp90 inhibition, and identified two potent small-molecule activators of human HSF1 within a chemical library of over 10,000 compounds [152]. These HSF1 activators (named HSF1A and HSF1C) are benzyl pyrazole derivatives (**Fig. 8**). The more potent compound, HSF1A, was then shown to activate HSF1 and induce Hsp70 in HeLa cells as well as in wild-type, but not in HSF1-deficient mouse embryonic fibroblasts. In contrast to the electrophilic HSF1 activators, pre-incubation with dithiothreitol had no effect on the inducer activity of HSF1A. A subsequent study has revealed that HSF1A functions by binding to and inhibiting the function of one of the negative regulators of HSF1, i.e. TRiC/CCT, an ATP-dependent chaperonin complex, thereby disrupting the interaction between TRiC/CCT and HSF1, and consequently leading to HSF1 activation [36].

Calamini et al. have conducted a mammalian cell-based high-throughput screen for HSR activation by cell-permeable small molecules in HeLa cells stably transfected with a luciferase reporter gene under the transcriptional control of the proximal human *HSP70.1* promoter sequence [153]. Approximately 900,000 compounds were screened, and ~200 small molecule activators of the HSR were found. Electrophilicity is a common feature among many, although not all, active compounds. A cyclohexanone derivative (named compound A1) containing the electrophilic α,β -unsaturated carbonyl groups is closely related to bis(benzylidene)acetone and bis(2-hydroxybenzylidene)acetone (**Fig. 8**), two structurally similar compounds, which however differ greatly in inducer potency [138]. Inducer potency correlates with sulfhydryl reactivity: compared to the hydroxylated analogue, bis(2-hydroxybenzylidene)acetone, a robust HSF1 activator [138], the parent compound bis(benzylidene)acetone reacts more slowly with the sulfhydryl groups of glutathione and dithiothreitol [154], and is essentially inactive as an Hsp70 inducer [138]. The electrophilic acetylenic *bis*(cyano enone) (\pm)-(4b*S*,8a*R*,10a*S*)-10a-ethynyl-4b,8,8-trimethyl-3,7-dioxo-3,4b,7,8,8a,9,10, 10a-octahydrophenanthrene-2,6-dicarbonitrile (TBE-31) (**Fig. 8**) that reacts readily and

reversibly with sulfhydryl groups [155], is another inducer of Hsp70, which is active at high nanomolar concentrations in wild-type, but not in HSF1-/- MEF cells [138].

The mildly electrophilic sulfoxythiocarbamate analogues of the isothiocyanate sulforaphane constitute another class of synthetic HSF1 activators.

Sulfoxythiocarbamate alkyne (STCA) (**Fig. 8**), which forms stable adducts with sulfhydryl groups [137], forms adducts with cysteine 412, cysteine 564, and cysteine 589/590 of recombinant Hsp90, and activates HSF1-mediated transcription of a luciferase reporter as well as of the endogenous Hsp70 in mammalian cells of many different types, including MEFs, HeLa, and MCF7 cells [139].

Pyrrolidinedithiocarbamate and 1,2-dithiole-3-thione are two other activators of HSF1-mediated induction of Hsp70 [156, 157], which have the ability to react with sulfhydryl groups. Activation of HSF1 and induction of the HSR has also been reported to occur upon exposure of cells to pro-electrophilic oxidizable diphenols; in this case, the corresponding oxidized electrophilic metabolites are the ultimate inducers [158].

Small Molecule Inhibitors of HSF1

Due to the ability of HSF1 to promote cancer cell survival and metastasis in animal models, its frequent activation in human tumors, and correlation with poor patient prognosis [159, 160], the discovery of small molecule HSF1 inhibitors and their development as anticancer drug candidates are actively being pursued [161]. Using a gel shift assay Nagai et al. [162] reported that the flavonoid quercetin (3,3',4',5,7-pentahydroxyflavone) (**Fig. 9**) inhibits the binding of HSF1 to its promoter HSE sequences without affecting the ability of the transcription factor to form trimers. The group further found that quercetin caused a decrease in the levels of phosphorylated HSF1, and that this decrease was more apparent in heat-shocked cells than in control cells. A later study confirmed that quercetin caused depletion of HSF1 and inhibition of heat shock protein expression in neuroblastoma cells, thereby increasing sensitivity to doxorubicin treatment [163]. More recently, it was shown that quercetin inhibits HSF1, downregulates Hsp70, and facilitates tumor radiofrequency ablation in rats [164]. Notably, quercetin inhibits many kinases, including JNK and p38 MAPK [165]. It is thus possible that through inhibition of these kinases, quercetin may lead to a

decrease in the activating phosphorylation modifications within HSF1, thereby decreasing its transcriptional activity.

Au et al. [166] have developed a high-content imaging-based screening assay for the identification of HSF1 inhibitors based on the quantification of the formation of HSF1/Hsp70 granules that form in HeLa cells after heat shock. Initially, the authors screened a library of 1,300 compounds and identified a compound of an undisclosed structure with an IC_{50} value of 80 nM, which was shown to inhibit HSF1 phosphorylation and decrease the expression levels of Hsp70 and Hsp90. Very recently, the same strategy was used to screen a library of 100,000 compounds, and a number of inhibitors were identified, among which the most potent compound, PW3405 (**Fig. 9**) had an IC_{50} value of 240 nM [167]. Immunoblotting analysis confirmed that, similar to quercetin, these compounds were able to inhibit the activating HSF1 phosphorylation at serine 326, suggesting that kinase inhibition may underlie their mechanism of action.

Small molecules that inhibit HSF1 without affecting the phosphorylation of the transcription factor have also been reported. One example is the phytochemical triptolide (**Fig. 9**), a diterpene triepoxide derived from *Tripterygium wilfordii*. Curiously, this is the same plant that is also a source of the HSF1 activator celastrol. At nanomolar concentrations, triptolide reversibly inhibits HSF1-dependent transcription in HeLa cells stably expressing the human *HSP70* promoter-driven luciferase reporter, and enhances apoptosis induced by severe heat shock [168]. Triptolide treatment prevents the inducible expression of heat shock genes by a mechanism that is not fully understood, but involves abrogation of the transactivation function of HSF1 without affecting trimer formation, hyperphosphorylation, or DNA binding [168]. In agreement with its HSF1 inhibitory activity, triptolide also decreases the formation of HSF1/Hsp70 granules that form in HeLa cells after heat shock [166].

By use of a luciferase reporter assay, Yoon et al. [169] screened a library of 6,230 compounds and identified a compound named KRIBB11 [N2-(1H-indazole-5-yl)-N6-methyl-3-nitropyridine-2,6-diamine] (**Fig. 9**) with an IC_{50} value of 1 μ M, which was shown to bind HSF1 and inhibit the HSF1-dependent recruitment of positive transcription elongation factor b (p-TEFb) to the *HSP70* promoter. Most recently, using an HSE-driven reporter-based chemical screen of more than 300,000 compounds in cells subjected to proteotoxic stress by treatment with the proteasome

inhibitor MG132, Santagata et al. [170] identified the protein translation initiation inhibitor rocaglate and a number of its analogs as inhibitors of HSF1 activation. The most potent compound had an IC_{50} value of 20 nM, and was named rohinitib (**Fig. 9**) (or RHT: Rocaglate, Heat Shock, Initiation of Translation Inhibitor). The levels of HSF1 in the cell did not change upon exposure to RHT. However, chromatin immunoprecipitation coupled with massively parallel DNA sequencing (ChIP-Seq) analysis revealed that RHT abolished HSF1 binding to DNA throughout the genome.

Most recently, 200,000 small molecules (consisting of 35,000 kinase-directed compounds and a diversity set of 165,000 compounds from the AstraZeneca collection) underwent a phenotypic screen in the U2OS human osteosarcoma tumour cell line treated with 17-allylamino-17-demethoxygeldanamycin (17-AAG), an Hsp90 inhibitor which triggers the HSR causing upregulation of Hsp70 [171]. The screen identified a 4,6-disubstituted pyrimidine derivative, and after optimization in an extensive structure-activity relation study based on the 4,6-pyrimidine scaffold, the resulting piperidine analog (**Fig. 9**) had an IC_{50} value of 15 nM. Interestingly, this compound was also a highly potent inhibitor of cyclin-dependent kinase 9 (CDK9). To test the possibility that CDK9 inhibition could be involved in inhibiting HSF1, two other structurally distinct validated CDK9 inhibitors were tested and found to be potent inhibitors of the HSF1-mediated HSR. One of these compounds, SNS-032 (**Fig. 9**), had been previously shown to inhibit HSF1-dependent induction of Hsp72 following treatment of A549 human non-small cell lung carcinoma cells with the Hsp90 inhibitor ganetespib [172]. It is currently unknown whether CDK9 affects the HSF1 activity directly, by catalyzing activating phosphorylation modifications within the transcription factor, or indirectly, in its capacity as a component of the positive transcription elongation factor b (P-TEFb), which stimulates transcription by phosphorylating RNA polymerase II.

Concluding Remarks

The efforts of many investigators have led to the current understanding of the existence of multiple layers of regulation of HSF1, including post-translational modifications (phosphorylation, sumoylation, and acetylation), proteasomal degradation, as well as the modulation of this transcription factor by small molecule activators and inhibitors. Because the function of HSF1 is dysregulated in a number

of human pathologies, including cardiovascular and neurodegenerative diseases and cancer, as well as during ageing, small molecule pharmacological modulators represent potential drug candidates. It should be pointed out that none of the known HSF1 modulators (activators or inhibitors) are specific, highlighting the fact that HSF1 does not operate in isolation, but in concert with other protein partners and stress-response transcription factors. One prominent example is transcription factor nuclear factor-erythroid 2 p45-related factor 2 (NRF2), which controls the gene expression of networks of antioxidant, drug-metabolizing, anti-inflammatory, and metabolic proteins, providing an interface between redox and intermediary metabolism [173]. A large number of studies suggest the existence of crosstalk between NRF2- and HSF1-regulated cytoprotective responses; these studies have been recently reviewed [174]. Global transcriptional profiling and proteomics analyses have consistently shown that, in addition to activating HSF1, most of the inducers discussed above, both naturally occurring as well as synthetic, also activate NRF2. It is thus very likely that together, the transcriptional programs orchestrated by HSF1 and NRF2 mediate the overall cytoprotective effects of these dual activators. Sulfhydryl reactivity is a common feature of these compounds and plays a critical role in the activation of both transcription factors, although the inducer concentrations that are required to activate HSF1 are generally higher than those that activate NRF2. The recognition of the importance of sulfhydryl reactivity of small molecule activators has been instrumental for elucidating the mechanisms by which HSF1 and NRF2 are regulated. Further research is needed to identify the precise points of crosstalk between these cytoprotective mechanisms and be able to determine suitable intervention strategies for their targeting for disease prevention and treatment.

Acknowledgments

We are very grateful to Cancer Research UK (C20953/A18644) and the BBSRC (BB/L01923X/1) for supporting our research.

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Figure Legends

Figure 1. Functional domain structure of HSF family members (HSF1-4) across different species. Shown are the DNA-binding domain (DBD, green), the leucine zipper domain 1-3 (LZ1-3, blue), the regulatory domain (RD, yellow), the leucine zipper 4 domain (LZ4, blue) and the C-terminal transactivation domain (CTAD, orange). The uncharacterized domains are colored in pink.

Figure 2. Molecular phylogenetic analysis of HSF family members by the Maximum Likelihood method. The evolutionary history was inferred by using the Maximum Likelihood method based on the JTT matrix-based model [175]. The tree with the highest log likelihood (-15403.2303) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 17 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 450 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 [176]. The mouse, rat, dog, human, bovine, and chicken HSFs are abbreviated with mHSF, rHSF, dHSF, hHSF, bHSF and cHSF, respectively. The HSF sequences were obtained from the UniProt database (uniprot.org). UniProt IDs: mHSF1-4 (P38532, P38533, D0VYS2 and Q9R0L1), rHSF(1-2 and 4) (Q63717, Q9R120 and A0A096MK39), dHSF4 (Q1HGE8), hHSF(1-2 and 4) (Q00613, Q03933 and Q9ULV5), bHSF(1-2 and 4) (Q08DJ8, A4FUA9 and F1MVT6) and cHSF(1-3) (P38529, P38530 and P38531).

Figure 3. The heat shock response. Various types of cellular stressors such as heat and proteotoxicity can activate the heat shock response by causing the release of the transcription factor HSF1, which in unstressed conditions is basally sequestered by the Hsp90 multichaperone complex that includes the co-chaperone Hsp70. In addition, the binding between the leucine zipper (LZ) domains LZ1-3 and LZ4 of HSF1 is disrupted, and the regulatory domain (RD) becomes hyperphosphorylated. The activated HSF1 enters the nucleus, homotrimerizes, and binds to the heat shock elements (HSEs) to induce the transcription of its target genes. HSF1 that is present in the nucleus also becomes activated upon stress and is able to homotrimerize to elicit the heat shock response. In the attenuation phase, Hsp70 present in the nucleus is able

to bind to the C-terminal transactivation domain (CTAD) of HSF1 to inhibit its function. During this time HSF1 is also dephosphorylated and shuttles out of the nucleus, resumes its inactivated form and is once again bound to the Hsp90 multichaperone complex.

Figure 4. Schematic diagram of the structure and posttranslational modifications of HSF1. The activating modifications (blue) are phosphorylation (Ph) modifications on serine residues 230, 326, and 419. The repressive modifications (red) on HSF1 are acetylation (Ac) on lysine 80, phosphorylation on Ser serine 121, 172, 303, 307 and 363 and sumoylation (Su) on lysine 298. The enzymes that catalyze these modifications are indicated beside the respective residue modifications; DBD, N-terminal DNA-binding domain; LZ, Leucine zippers LZ1-3 and LZ4; RD, Regulatory domain; CTAD, C-terminal transactivation domain. Trimerization of HSF1 occurs at the LZ1-3 region, which during basal conditions is negatively regulated by LZ4. In addition, under unstressed conditions, the CTAD is negatively regulated by the RD.

Figure 5. Crystal Structure of the DNA-binding domain (DBD) of human HSF1 (amino acids 10-123). The domain is colored according to the secondary structure of the protein where the alpha helices and beta sheets are represented by red and yellow, respectively. Amino acid residues lysine 80 (K80) and serine 121 (S121) are represented in blue. Figure drawn with Pymol using the PDB entry 2LDU.

Figure 6. Endogenous small molecule activators of HSF1.

Figure 7. Phytochemical activators of HSF1.

Figure 8. Synthetic small molecule activators of HSF1.

Figure 9. Small molecule inhibitors of HSF1.

Figure 1

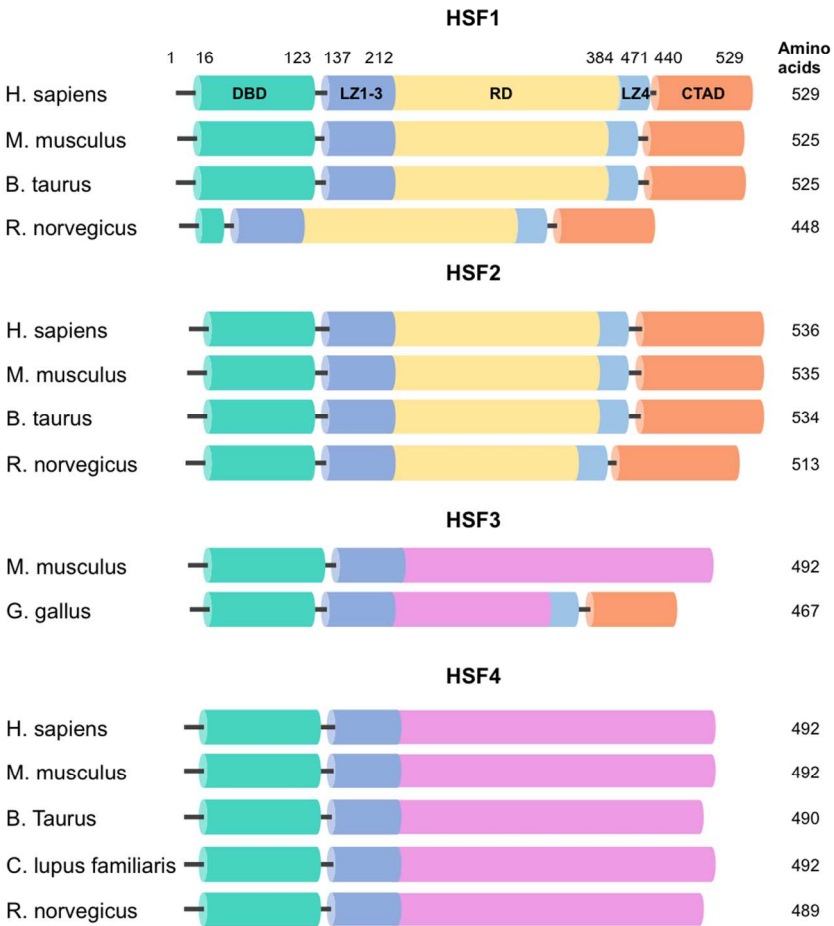


Figure 1

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Figure 2

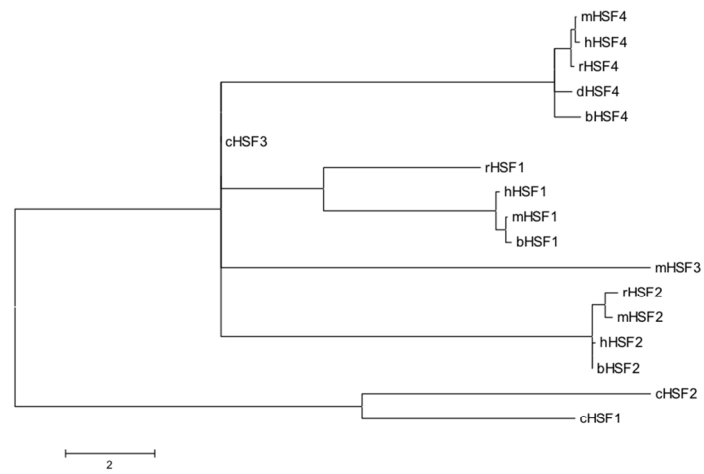


Figure 2

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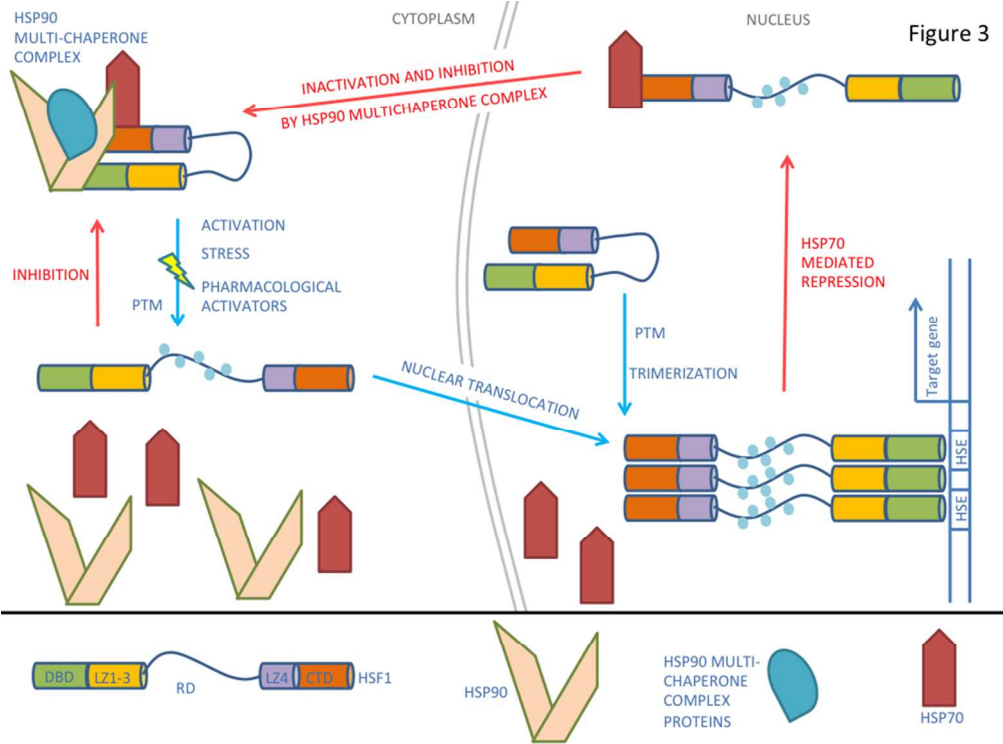


Figure 3

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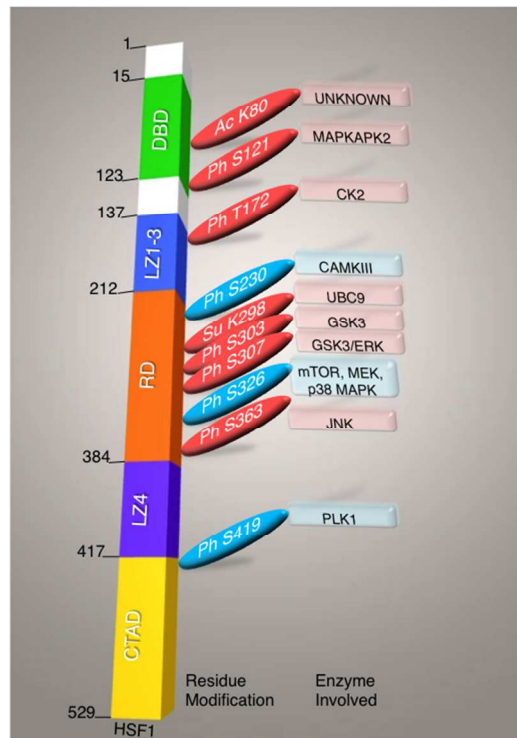


Figure 4

Figure 4

352x264mm (72 x 72 DPI)

Figure 5

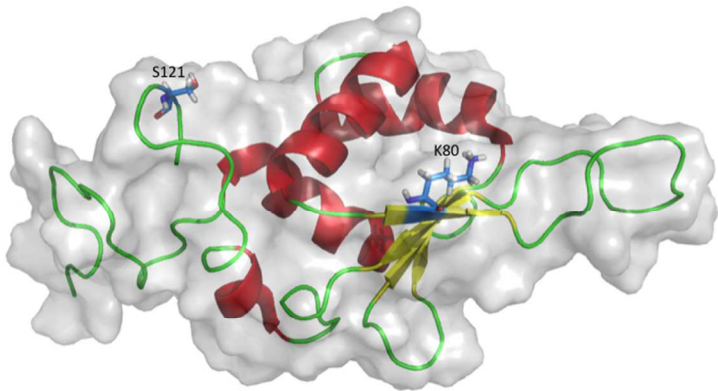


Figure 5

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Figure 6

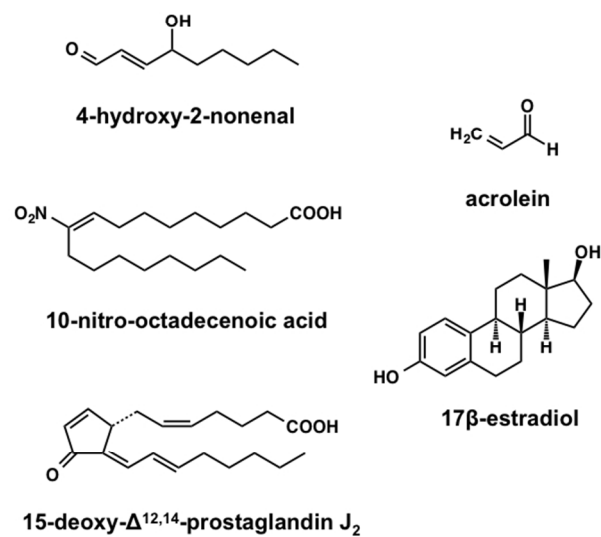


Figure 6

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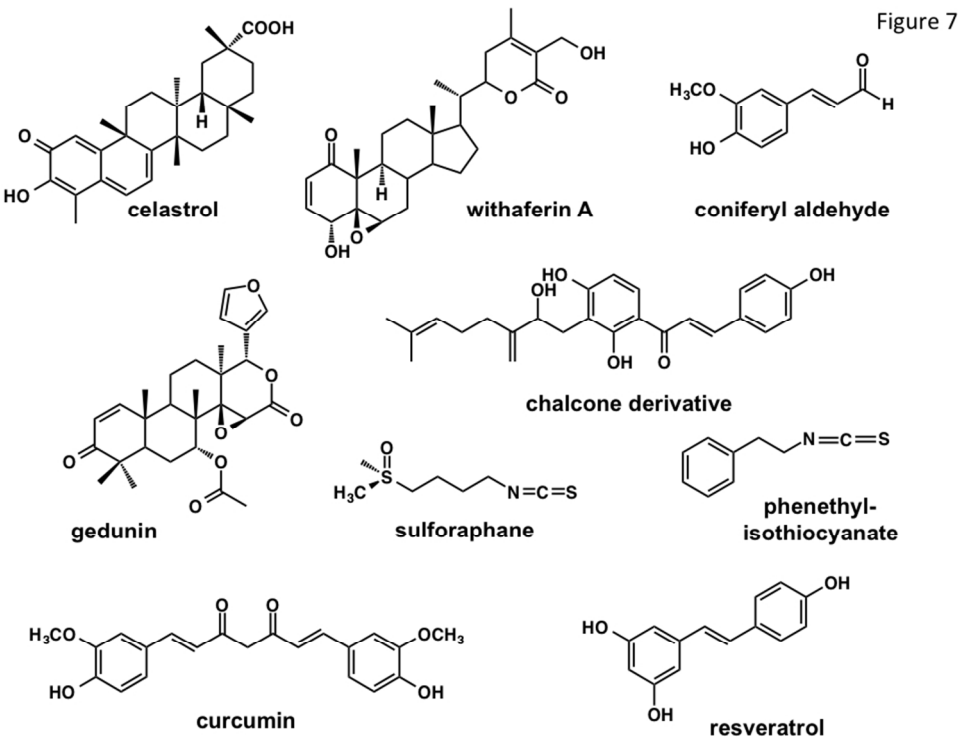


Figure 7

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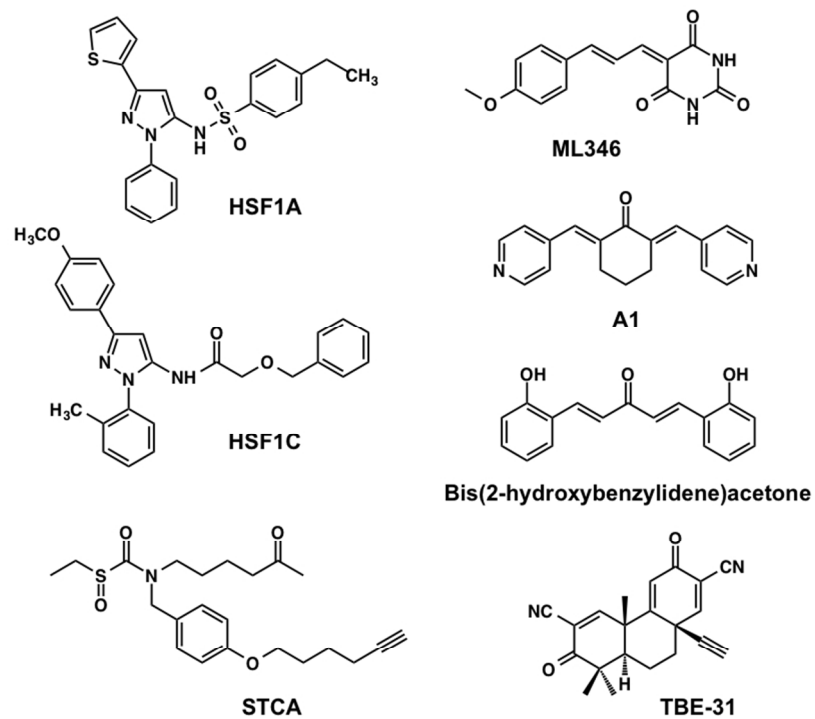


Figure 8

Figure 8

352x264mm (72 x 72 DPI)

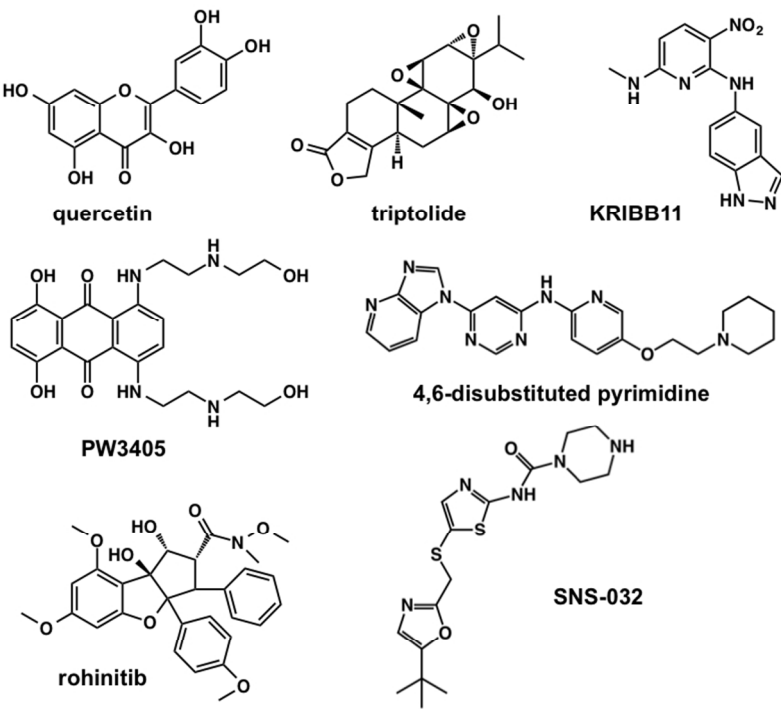


Figure 9

Figure 9

352x264mm (72 x 72 DPI)